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# Milk protein fraction profile in Simmental cows and its relationships with milk quality and physiological factors

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**Abstract:** *Milk protein fractions determine the technological and nutritional value of milk. We characterised the protein fraction profile of milk from 93 Romanian Simmental cows by microfluidic capillary electrophoresis and related it to milk composition, udder health and physiological factors. After run-level quality control, α-casein (31.9%) and β-casein (31.8%) were the largest fractions, followed by β-lactoglobulin (12.0%), κ-casein (9.2%) and α-lactalbumin (3.8%). Higher somatic cell count (SCC) was associated with a lower β-casein proportion (r = −0.45, the only association surviving false-discovery-rate control), a higher α-lactalbumin proportion (r = 0.31) and lower lactose (r = −0.59), consistent with casein proteolysis.*

## • Introduction

- Bovine milk protein is ≈ 80% caseins ($\alpha_{S1}$, $\alpha_{S2}$, β, κ) and ≈ 20% whey proteins, mainly β-lactoglobulin (β-LG) and α-lactalbumin (α-LA).
- Their proportions affect coagulation, cheese yield and nutritional value.
- High somatic cell count (SCC) raises plasmin activity, which degrades caseins, mainly β-casein [1, 2].
- No fraction profile has been published for Romanian Simmental.

**Aim:** describe the five major protein fractions in Romanian Simmental milk and relate them to milk composition, udder health and physiological factors.

## • Material and method

93 lactating Romanian Simmental cows, research herd of SCDCB Arad; one individual sample per cow at the morning milking.

| 93 | 4.0 | 241 |
|---|---|---|
| cows | median parity | median days in milk |

- **Mid-infrared spectroscopy:** fat, protein, casein, lactose, urea, BHB, acetone.
- **Flow cytometry** (Fossomatic 7 DC): SCC and differential SCC.
- **Microfluidic electrophoresis** (Agilent 2100 Bioanalyzer, Protein 80 kit) [3]: α-, β-, κ-casein, β-LG, α-LA as % of total peak area.
- **Quality control (QC) of every run:** chip 8 (10 runs) and 2 other runs excluded for misassigned peaks; α- and β-casein set to missing in 30 runs where one peak carried both labels (Fig. 1).
- **Statistics:** $\log_{10}$ SCC; Pearson and Spearman correlations with false-discovery-rate control (35 tests); multiple regression on days in milk (DIM), parity, $\log_{10}$ SCC and urea, with and without chip.

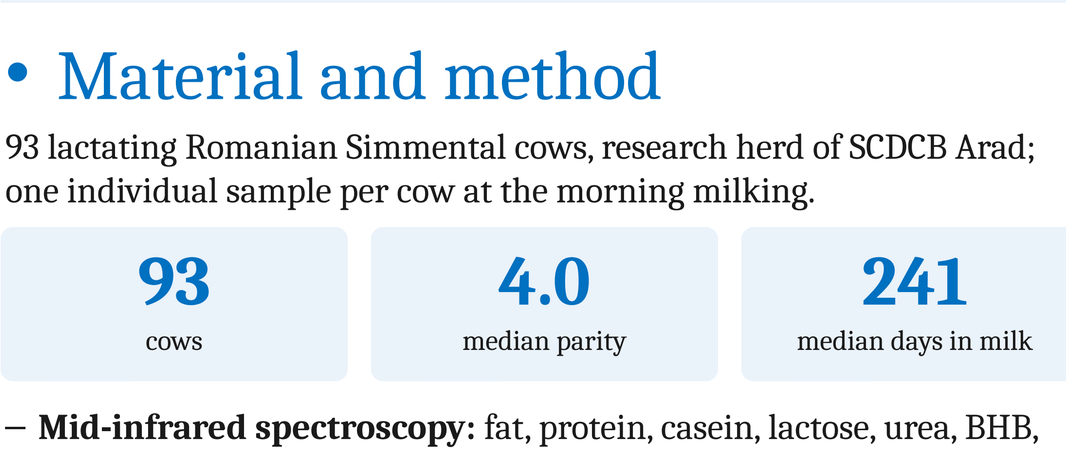



**Fig. 1.** Each fraction in every run, by chip. Chip 8 (shaded) had shifted migration times and impossible values (β-LG ≤ 0.9%, β-CN ≤ 1.5%, κ-CN ≥ 29.4%).

## • Results and discussions

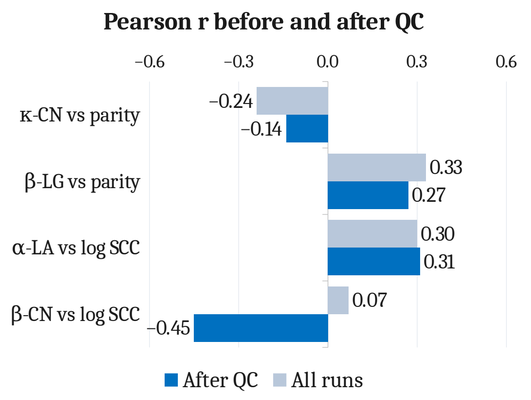



**Fig. 2.** Before QC, the chip explained 87% of β-CN variance and created spurious parity effects.

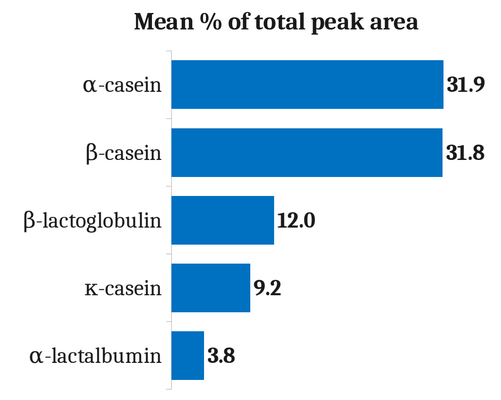


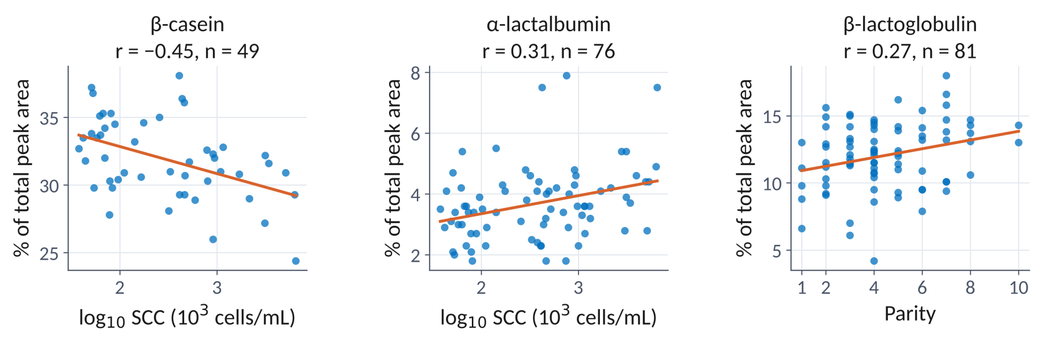

**Fig. 3.** Within caseins α 44.6%, β 44.0%, κ 11.4%; β-LG 76.4% of whey; close to RP-HPLC in Simmental [4].

| | DIM | Parity | log SCC | DSCC | Urea | Fat | Casein |
|---|---|---|---|---|---|---|---|
| **α-casein** | 0.05 | **−0.31** | **−0.34** | −0.18 | 0.19 | 0.05 | 0.10 |
| **β-casein** | −0.27 | −0.11 | **−0.45†** | **−0.29** | 0.09 | 0.16 | −0.01 |
| **β-lactoglobulin** | 0.09 | **0.27** | 0.18 | 0.09 | 0.04 | −0.08 | 0.05 |
| **κ-casein** | 0.00 | −0.14 | −0.01 | 0.02 | −0.01 | 0.11 | 0.19 |
| **α-lactalbumin** | 0.09 | 0.12 | **0.31** | 0.08 | 0.11 | 0.07 | −0.02 |

**Table 1.** Pearson r after QC (n = 45–81). Bold: p < 0.05; †: false discovery rate < 0.05. Blue: negative; orange: positive.

High SCC → Plasmin activity rises → β-casein cleaved to γ-caseins → **Lower β-CN share; higher α-LA share**

Plasmin cleaves β-casein into γ-caseins and proteose peptones, as in subclinical mastitis [1, 2]; shares are relative, so a smaller β-CN peak raises the others.




**−1.83 per $\log_{10}$ SCC**
p = 0.012 (with chip 0.059)

**+0.56 per $\log_{10}$ SCC**
p = 0.028 (with chip 0.014)

**+0.33 per lactation**
p = 0.033 (with chip 0.056)

**Fig. 4.** Main associations after QC; regression coefficients (percentage points) adjusted for DIM, parity, $\log_{10}$ SCC and urea.

## • Conclusions

- α- and β-casein dominate Romanian Simmental milk; the profile is close to RP-HPLC data for Simmental.
- Higher SCC: less β-casein, a higher α-lactalbumin share and less lactose, consistent with casein proteolysis.
- Parity had only a weak effect on β-lactoglobulin; stage of lactation had none.
- Microfluidic electrophoresis needs run-level quality control of peak assignment.

**References**
[1] Urech E, et al. J Dairy Sci 1999;82:2402–2411. [2] Bisutti V, et al. J Dairy Sci 2022;105:6447–6459. [3] Nitsche R. Agilent Application Note 5990-8125EN, 2016. [4] Bonfatti V, et al. J Dairy Sci 2010;93:3797–3808.

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